# FURTHER STUDIES IN ARSPHENAMINE HYPERSENSITIVENESS IN GUINEA PIGS

## III. Investigations on the Chemical Specificity of Skin Hypersensitiveness of Guinea Pigs to Old Arsphenamine[1, 2]

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Examinations on the chemical specificity of skin hypersensitiveness of guinea pigs to arsphenamines have been made by Sulzberger and F. A. Simon and other investigators (see below). Additional detailed studies of this question seemed to be indicated to the present author, especially in relation to former similar examinations in man.

### REVIEW AND DISCUSSION OF THE LITERATURE

Frei and R. L. Mayer (1) applied a series of 32 different substances intracutaneously to three male patients who had recovered from generalized exfoliative neosalvarsan dermatitis and to six normal persons.[3] All three cases of dermatitis displayed hypersensitiveness of varying degree not only to arsphenamines but to one member of another group of aromatic

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[2] Former articles in this series are: "Further Studies in Arsphenamine Hypersensitiveness in Guinea Pigs. I. Cutaneous and Anaphylactic Responses to Old Arsphenamine and to Neoarsphenamine after Sensitization with Old Arsphenamine," by Wilhelm Frei and Marion B. Sulzberger, Jour. Invest. Derm. **1**: 191, June, 1938; and "Further Studies in Arsphenamine Hypersensitiveness in Guinea Pigs. II. Attempts at Experimental Specific Sensitization of Guinea Pigs to Quinine, to Acetyl Salicylic Acid, and to Barbital, with and without Preceding or Concomitant Arsphenamine Sensitization." Jour. Invest. Derm., **4**: 111, April, 1941.

[3] Cannon and Karelitz (2) observed a high percentage of non-specific inflammations in intracutaneous arsphenamine tests in man, obtaining most of the inflammatory reactions with dilutions of 1:100 (neoarsphenamine) and 1:200 (neutralized old arsphenamine, silver arsphenamine). The same neoarsphenamine concentration was employed by Olin (3) in recent examinations.

Frei and R. L. Mayer, influenced by their own previous experiences, did not use stronger neoarsphenamine concentrations than 0.15:100 for intracutaneous testing, and prepared their dilutions with physiologic saline solution instead of with distilled water. They read and evaluated the tests, like all tests with allergens of originally irritating character, by *actually comparing* the reactions of dermatitis cases and control persons, tested *at the same time* with the same freshly prepared solutions and examined after 24 and 48 hours.

The present author, who had used intracutaneous arsphenamine tests in man almost exclusively for scientific and not for practical purposes, later abandoned them entirely because of the possibility of sensitization or activation respectively—on this point being in accord with Moore, Woo, Robinson and Gay (4); Klauder (5); Cannon and Karelitz (2); Schoch (6b), and other investigators.

trivalent arsenicals, an arsine oxide, and also, to certain aromatic pentavalent arsenicals. Two of the patients gave positive tests even to one of three aliphatic arsenical compounds. None of them reacted specifically to inorganic tri- or pentavalent arsenicals, or to any compound of the benzene group, more or less related to arsphenamine, but not containing arsenic. According to Frei and R. L. Mayer, their own findings did not indicate that all cases of generalized exfoliative arsphenamine dermatitis must have the same chemical basis; as a matter of fact, some differences were also observed even among these three cases. (For further details, discussion and literature, see Frei and R. L. Mayer; see also Garnier (7); Moore, Woo, Robinson and Gay (4); Puente and Cordiviola (8); Cannon and Karelitz (2); et al.)

After discovery of the method of artificial specific sensitization of man to arsphenamine (Frei and R. L. Mayer (1); Frei (9a); Nathan and Munk (10a); Kaplun and Moreinis (11); Ensbruner (12); Hanaoka (13)); Frei[4] (9a) in a short preliminary experiment with neosalvarsan (Hoechst), and Nathan and Grundmann (10b), in more extended series with myosalvarsan (Hoechst), tested *persons who were artificially sensitized* to these preparations, comparing them with normal controls. The results of these tests were similar to, though not entirely identical with the observations obtained in generalized exfoliative neosalvarsan dermatitis by Frei and R. L. Mayer.

All persons reacted specifically to various kinds of arsphenamine, most of them to arsine oxide, some to a lesser degree to diverse aromatic pentavalent arsenicals, and none to aliphatic or inorganic arsenicals, or to aromatic compounds without arsenic (see Nathan and Grundmann (10b)).

It should also be noted that in recent investigations of Chargin and Leifer (17), activation of *fixed eruptions due to arsphenamines* was produced not only by various arsphenamines but also in many cases by arsine oxide and by some aromatic pentavalent arsenical compounds.

When the method of artificial sensitization to arsphenamines was applied to guinea pigs (Frei (9b); Sulzberger (18a); et al.), intracutaneous testing for chemical specificity was performed in these *animals* (Sulzberger and F. A. Simon (18d); Cormia (14a); Miescher and Schnitzer (19) et al.). Here specific reactions were obtained *only with various arsphenamines*, but not with aromatic pentavalent arsenicals. Williamson (20) mentioned positive reactions elicited by mapharsen, an arsine oxide.

The question arises as to whether *variations of qualitative or of quantitative character* were responsible for these differences in the results obtained.

There is no doubt that qualitative differences exist between the allergic state of man afflicted with generalized exfoliative *arsphenamine dermatitis* and that of man *artificially sensitized* by intracutaneous injections of arsphenamine. For, hypersensitiveness of the latter kind predisposes clinically mainly to eruptions of the erythemato-morbilliform-urticarial type and not to those of the dermatitis type (Nathan (10c); Kaplun and Moreinis (11); discussed by Frei (9c)).[5]

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[4] Several American investigators, studying the sensitization of man to arsphenamines by intracutaneous injections, obtained either 1) different results (Moore, Woo, Robinson and Gay (4)) or 2) negative results (Cannon and Karelitz (2); Schoch (6b)). Cormia (14b) offers the hypothesis that this "common failure to produce sensitization experimentally in human beings may be partially due to a failure to duplicate collateral factors which have been found important in animal experimentation." (Sulzberger and Mayer; Sulzberger and Simon.) There are, however, other possible explanations also. On the question of sensitization to arsphenamines by patch test see Beerman (15) and Stokes (16).

[5] Frei at the time of his discovery of the method of artificial sensitization of man to arsphenamine by intracutaneous injections, first believed that the hypersensitiveness produced by this procedure was identical with the hypersensitiveness of cases of generalized exfoliative arsphenamine dermatitis. Later, he changed his opinion as a result of the experiments conducted in the meantime by the above-named investigators.

On the other hand, there is a strong similarity of skin symptoms in *man* artificially sensitized to arsphenamines by intracutaneous injections compared to those in *guinea pigs* treated in the same way. In both man and guinea pigs flare-ups may appear on the site of the first injections, as a sign of acquired hypersensitiveness. Such hypersensitiveness may also develop without previous flare-up; this happens frequently in animals, rarely in man. Furthermore, in the first extensive experiments by Frei (9b) on guinea pigs, distinct erythematous macules were observed in some of the most strongly sensitized animals, when reinjected intracardially nine or ten days after intracutaneous sensitization to neosalvarsan (Hoechst). These macules were localized mainly but not exclusively in the surroundings of the flare-ups and might, to a certain degree, be compared with the morbilliform erythemas described by Nathan (10c) in man sensitized to myosalvarsan (Hoechst). Kaplun and Moreinis (11) even observed universal morbilliform exanthemas in one of their guinea pig series.

In contrast to these close relationships, it is doubtful whether the *anaphylactic type* of arsphenamine hypersensitiveness in guinea pigs is based on the same immunological mechanism as the cutaneous type, although both types may develop after a single intracutaneous injection of old arsphenamine. (Landsteiner and Jacobs (21); Frei and Sulzberger (22).) The reason for this doubt is that animals sensitized by old arsphenamine give strong skin reactions to old arsphenamine as well as to neoarsphenamine, but respond with anaphylactic symptoms only to the first compound (Frei and Sulzberger (22)). This argumentation supports the opinion expressed by Cormia (14c) as a result of his findings in recent experiments.

The other possibility mentioned before is that *variations of quantitative character* were the cause of the different results obtained in skin testing of the various groups. Although a series of three cases of generalized exfoliative neosalvarsan dermatitis is rather small for subdividing, it may be mentioned that the two severe cases gave not only stronger reactions to arsphenamines than the mild one, but reacted to a greater number of pentavalent aromatic arsenicals and, in contrast to the mild case, also reacted to one of the aliphatic arsenicals. This scanty evidence, however, increases in significance because of the fact that analogous observations have been made by Nathan and Grundmann (10b) on persons artificially sensitized to myosalvarsan. Of their nine cases, the one which showed the highest degree of allergy toward arsphenamines was also the only one to react to all three of the aromatic pentavalent arsenicals tested. Such observations indicate that in arsphenamine hypersensitiveness the *intensity of the allergic state* has some influence on the range of positive reactions.[6]

There is another quantitative factor too, which one must not neglect. Frei and R. L. Mayer, whose tests demonstrated a somewhat broader base of sensitivity than those of Nathan and Grundmann, frequently *increased the concentration* of their test substances when the first reactions were negative. Nathan and Grundmann used only a single concentration, corresponding in weight either to their myosalvarsan concentration or to one-tenth of this amount.

The possibility exists that *the same quantitative factors were responsible for the small range of positive reactions in guinea pigs* sensitized to arsphenamines. The hypersensitiveness produced in guinea pigs by neoarsphenamine or some other arsphenamine compounds is sometimes rather low, especially under conditions explored by Sulzberger and co-workers (23). The only exception is old arsphenamine, which was not employed in those experiments because its strong sensitizing power was not known at that time. Furthermore, the test substances used in the former experiments on guinea pigs were mostly applied in one single concentration, equivalent, either in arsenic content, or in weight, to the customary neo-

[6] See also recent examinations of Schoch, Alexander and Long (6c): Mild cases of neoarsphenamine dermatitis tolerated mapharsen treatment after recovery and gave negative patch tests to mapharsen. Severe cases exhibited recurrences of dermatitis after very small mapharsen doses and reacted positively to mapharsen patch tests.

arsphenamine concentration (0.15:100) introduced by Frei for tests in man and guinea pigs sensitized to this preparation *itself*. The concentrations of substances more or less related to the sensitizing compound were not increased in those guinea pig experiments when specific reactions failed to appear. They were occasionally decreased when disturbing non-specific reactions were produced by this concentration.

Observations on artificial conjugated antigens have shown that "antibodies react most strongly to the homologous antigen, but also regularly, with graded affinity, on chemically related substances" (Landsteiner (24)). Such gradations may, *to some extent*, also play a rôle in arsphenamine hypersensitiveness, and should be taken into consideration in skin testing for specificity. If one wishes to go deeper into the chemical relationship of the arsphenamine allergy, it would be advisable, therefore, *to increase the hypersensitiveness of the test animals, as well as the concentration of the test substances*, as much as possible. Distinct non-specific irritations should, of course, be avoided.

## GENERAL PROCEDURE

Therefore, in the following experiments on guinea pigs, *old arsphenamine*, as the strongest available allergen of the arsphenamine group, was employed for sensitization, and the *highest, well-tolerated concentrations* of the various test substances were applied for intracutaneous testing. In the case of most of the substances, the initial concentration used for testing was either equimolar to the customary test concentration of old arsphenamine (0.15:100) or was based on former experiences of the author or other investigators. If the first injection of a test substance did *not* give a positive result and, on the other hand, was *not* followed by any non-specific irritation, the concentration was increased until the non-specific inflammatory effect obtained in the control animals had about the same low grade as the mild primary reaction of non-sensitized guinea pigs to the above mentioned test concentration of old arsphenamine. If the irritating effect was stronger, the concentration was decreased. Table 1 demonstrates this procedure in the case of *sodium cacodylate*, tested in control animals.

The table demonstrates, at the same time, a difficulty frequently encountered in these experiments. On two occasions, ten per cent solutions of sodium cacodylate were applied to non-sensitized guinea pigs. They produced a mild irritation one day, and a very strong one, with central necrosis, another day. This *inconstancy of the irritating effect* was still more conspicuous with the use of some other arsenic preparations, for example, mapharsen, sodium arsenate and especially, sodium arsenite. Cormia (14a) discovered that the same concentration of potassium arsenite, which in experiments of Sulzberger and F. A. Simon (18d) produced a severe irritating effect and therefore, had to be reduced by these investigators, was well tolerated in his experiments. He believed "that the present group of pigs either had comparatively insensitive skins, or that the preparation itself is a varying factor." Of course, these two conditions may play a role in the interpretation of this phenomenon. However, one can also find inconstancies of the irritating effects in treating members of one batch of guinea pigs with the same brand of sodium arsenite on different days. Furthermore, the same control animals which reacted insignificantly to one intracutaneous injection of sodium arsenite have, several days later, reacted to another injection of the same strength with necrosis. The impression arose that this

phenomenon might also be connected with certain *weather conditions*. Hot weather, perhaps especially when accompanied by high humidity, seems to increase the necrotizing effect of these arsenic preparations on the skin of guinea pigs. This would be in accordance with the experience of the present author, that, in human therapy, disturbances produced by some arsenic preparations, for instance, by *solutio Fowleri*, are more frequent in summer than in other seasons.

TABLE 1

*Sodium cacodylate, applied in increasing concentrations intracutaneously to non-sensitized guinea pigs*

Experiments of former investigators have demonstrated that low concentrations of sodium cacodylate (0.087:100) do not elicit any allergic skin reaction in guinea pigs sensitized to arsphenamine (neoarsphenamine). On the other hand, one was led to expect from the experiences in human therapy, that sodium cacodylate might be used in much higher concentrations without damaging the skin. Therefore, skin testing of control animals with this preparation was done with concentrations increasing gradually from one to ten per cent. Finally, a solution of eight per cent was chosen for allergy tests—a concentration ninety-three times higher than that used by previous investigators. Intracutaneous injections of this high concentration, although accompanied by visible signs of pain, were followed only by very insignificant inflammation in control animals.

Solutions were prepared in distilled water immediately before intracutaneous injections. Three to six non-sensitized guinea pigs were used for each solution. The injected amount was 0.1 c.c. Reactions were read after one and two days.

The following terms are used in the table:

*None:* No visible irritation was produced.

*Insignificant:* Irritation was less pronounced than the mild irritation produced by the test concentration of 0.15 per cent of old arsphenamine in non-sensitized guinea pigs.

*Moderate:* Irritation was equal to that produced by 0.15 per cent of old arsphenamine.

*Strong:* Irritation was stronger than this irritation.

| Concentration of sodium cacodylate in per cent..... | 1 | 1.5 | 2 | 5 | 6.5 | 8 | 10 | 10 |
|---|---|---|---|---|---|---|---|---|
| Irritating effect on the skin of non-sensitized guinea pigs..... | None | None | None | Insignificant | Insignificant | Moderate | Moderate | Strong |

The preceding has set forth the principles upon which the following investigations on specificity of arsphenamine hypersensitiveness were based.

## EXPERIMENTAL DATA

A *first series* of experiments on guinea pigs sensitized to old arsphenamine, and on non-sensitized controls, was made in order to obtain a general survey of the field. The results are listed in table 2.

*In a second series* of experiments on guinea pigs sensitized to old arsphenamine, and on non-sensitized controls, intracutaneous testing of several of the sub-

TABLE 2

*Tests on guinea pigs sensitized to old arsphenamine, and on non-sensitized controls*

White virgin guinea pigs of 300 to 500 g. were used for sensitization to old arsphenamine. Sensitization was effected by a single intracutaneous injection of 0.1 c.c. of a 0.15 per cent aqueous solution of old arsphenamine Winthrop. The injections were followed by small inflammatory, pale pink, slightly elevated lesions of four to five mm. in diameter, which diminished or disappeared after a few days and were for the most part, replaced by a so-called flare-up. All of the animals injected in this series proved sensitized to old arsphenamine when reinjected with a solution of the original strength after four weeks.

The test substances, listed below, were dissolved in distilled water immediately before use. Four to six sensitized animals, and three non-sensitized guinea pigs of one batch were then injected intracutaneously, at the same time, with 0.1 c.c. of each solution. In some instances, as in mapharsen, tryparsamide, sodium cacodylate, sodium arsenate and sodium arsenite, testing was repeated three to five times in various concentrations. Reactions were read after one and two days.

The following signs are used in the table:

++ Strong reactions were observed in sensitized guinea pigs, consisting of large inflammatory papules with central necrosis.

++−+ Comparatively strong reactions were observed, consisting of somewhat smaller papules, accompanied by central necrosis in some of the sensitized animals.

\+ Reactions were less than those previously mentioned and without central necrosis, but definitely stronger than the small inflammatory lesions of the control animals.

(+) Reactions of the majority of sensitized animals were slightly stronger than those of the non-sensitized ones.

± No definite decision could be made as to whether there were differences in the reactions of sensitized and non-sensitized animals.

− No differences were observed in the reactions of sensitized and non-sensitized animals.

| SUBSTANCE | CONCENTRATION | RESULT |
|---|---|---|
| | *per cent* | |
| Old arsphenamine | 0.15 | ++ |
| Neoarsphenamine | 0.15 | ++ |
| Sulfarsphenamine | 0.25 | ++−+ |
| Silver arsphenamine | 0.08 | ++−+ |
| Mapharsen | 0.022 | (+) |
| Tryparsamide | 4.0 | (+) |
| Sodium cacodylate | 6.5 | ± |
| Sodium arsenite | 0.035 | − |
| Sodium arsenate | 0.2 | ± |
| o-aminophenol hydrochloride | 0.2 | − |
| p-aminophenol hydrochloride | 0.2 | − |
| p-aminoazobenzene hydrochloride | Almost 0.1 (saturated solution) | − |
| Azobenzene | Saturated in boiling water | − |
| Acetyl salicylic acid | About 0.17 (half saturated solution) | − |
| Barbital | About 0.38 (half saturated solution) | − |
| Quinine hydrochloride | 0.2 | − |

stances, examined in the first series, was repeated, and other preparations were added to the list. In these experiments testing was done under special precautions (see below) and on a greater number of animals than was used in the first series.

## ANALYSIS OF RESULTS

According to the results listed in tables 2 and 3, guinea pigs sensitized with old arsphenamine, display skin hypersensitiveness not only to the sensitizing preparation, but at the same time, to a greater or lesser degree, to other *arsphenamines*. The majority of tested animals also gave slightly positive reactions to a member of another group of aromatic trivalent arsenicals, to mapharsen—an *arsine oxide*. Furthermore, positive reactions were produced by three *aromatic pentavalent arsenicals*, namely, atoxyl, tryparsamide, and sodium p-hydroxyphenylarsonate, though to a lesser degree than by arsphenamines. These positive reactions to aromatic pentavalent arsenicals were obtained only when higher concentrations were used than had been used in experiments of former investigators, whereas concentrations corresponding to those formerly applied, gave the same negative results as previously. This fact permits the interpretation that it was not so much the change to old arsphenamine as sensitizing agent, as the *increase of the test concentrations* that was responsible for these positive results.

The reactions of sensitized guinea pigs were questionable or negative to three *aliphatic arsenicals*, sodium cacodylate, solarson and n-propylarsonic acid, and to the tri- and pentavalent *inorganic arsenicals*, sodium arsenite and sodium arsenate. Also seven *aromatic chemicals, not containing arsenic*, were examined; among these were four azo compounds and amino products of the benzene group as substances related to the organic part of arsphenamine, and two heterocyclic compounds, quinine hydrochloride and barbital, as substances not related to arsphenamine. In contrast to the benzene derivatives containing arsenic, *none* of the azo and amino products without arsenic gave any trace of a positive reaction; nor did any of the other organic substances tested.

The report thus far, contains some facts already *established* by previous investigations, and some others hitherto *not known*. That sensitization of guinea pigs to one arsphenamine preparation produces hypersensitiveness to various members of the arsphenamine group has been demonstrated by experiments of R. L. Mayer (25); Sulzberger and F. A. Simon (18d); Frei and Sulzberger (22); and Miescher and Schnitzer (19). That this hypersensitiveness extends to mapharsen has been mentioned by Williamson (20). That it does not extend to inorganic arsenicals has been stated by Sulzberger and F. A. Simon and by Cormia (14a). On the other hand, specific skin reactions to aromatic pentavalent arsenicals have not been produced previously in guinea pigs sensitized to arsphenamine. Aliphatic arsenicals have been tested only in the case of a single preparation, sodium cacodylate, and in very low concentrations of that preparation. Also organic compounds related to arsphenamine, but not containing arsenic, have not yet been examined to any noticeable extent. Sulzberger and

TABLE 3

*Further tests on guinea pigs sensitized to old arsphenamine, and on non-sensitized control animals*

White virgin guinea pigs of about 400 g. were sensitized to old arsphenamine by a single intracutaneous injection of 0.1 c.c. of a 0.15 per cent solution of arsphenamine Winthrop in saline solution. The injections were followed by slightly elevated pale pink papules of three to four mm. in diameter and, a few days later, by flare-ups in all cases. All animals proved to be sensitized to old arsphenamine when reinjected with a solution of the original brand and strength after four weeks. Both arsphenamine injections were done on the backs of the animals.

The test substances were dissolved in *saline solution* (see Frei and R. L. Mayer; Frei; Frei and Sulzberger), since under this condition their irritating effect was less pronounced than in aqueous solution, and, consequently, higher concentrations could be applied to the animals. Only in very high, hypertonic concentrations, as prepared from tryparsamide and sodium cacodylate, was there no difference in the irritating effect, either with or without the addition of sodium chloride. In these cases, aqueous, as well as saline solutions were used for testing. All solutions were freshly prepared from sterilized *triple distilled water*. Only glassware, syringes, and cannulas (26 gauge) which never before had come *in contact with arsphenamines* were employed in the experiments of this series (see Schoch[6a]).

Each solution was tested intracutaneously in one batch of nine sensitized and ten non-sensitized animals at the same time. Injections were performed on the flanks, at least one day after very exact shaving. The amount injected was 0.1 c.c. Reactions were read after one and two days.

The signs used to indicate the results in this table are the same as those used in Table 2.

At the end of this experiment, all animals were tested to old arsphenamine. The animals previously sensitized to this preparation gave positive, and the control animals negative reactions.

| SUBSTANCE | CONCENTRATION | RESULT |
|---|---|---|
| | *per cent* | |
| Old arsphenamine | 0.15 | ++ |
| Atoxyl | 0.15 | − |
| Atoxyl | 1.0 | + |
| Atoxyl | 1.2 | + |
| Tryparsamide | 0.2 | − |
| Tryparsamide | 9.0 | + |
| Tryparsamide* | 9.0 | + |
| Sodium p-hydroxyphenylarsonate | 0.1 | − |
| Sodium p-hydroxyphenylarsonate | Almost 1.0 (hot saturated solution) | (+) |
| Sodium p-hydroxyphenylarsonate* | Almost 1.0 (hot saturated solution) | (+) |
| Sodium cacodylate | 6.5 | − |
| Sodium cacodylate | 8.0 | ± |
| Sodium cacodylate* | 8.0 | − |
| Solarson (chlorarsenol)† | 1.0 | − |
| n-propylarsonic acid | Almost 0.5 (hot saturated solution) | ± |
| Sodium arsenite | 0.17 | − |
| Sodium arsenate | 1.2 | − |
| o-aminophenol hydrochloride | 0.5 | − |
| p-aminophenol hydrochloride | 0.6 | − |
| p-aminoazobenzene hydrochloride‡ | 0.12 | − |
| Azobenzene‡ | 0.032 | − |

* Solution was prepared in distilled water instead of in saline solution.

† Commercially prepared solutions were used.

‡ Solutions were prepared by diluting two per cent alcoholic solutions in saline.

F. A. Simon have already stressed the necessity of examinations of the latter kind. They also obtained negative results in testing aminopyrine on guinea pigs sensitized to neoarsphenamine.

The results of the present experiments on guinea pigs are similar to those obtained by Frei and R. L. Mayer in examinations on *human beings who had recovered from generalized exfoliative neosalvarsan dermatitis.* The conclusion drawn by Frei and Mayer in 1927 with regard to their cases is, to a great extent, also valid for the skin hypersensitiveness of guinea pigs to old arsphenamine. This conclusion was "The hypersensitiveness was neither directed toward arsenic nor toward the organic part of arsphenamine, nor exclusively toward arsphenamines, but was directed toward organic arsenical compounds" (translation). A single different finding was that in guinea pigs no aliphatic arsenical gave a positive reaction. On this, as well as on other points, the results on guinea pigs are in complete accordance with the findings of Nathan and Grundmann on *man sensitized by intracutaneous injections of myosalvarsan.* Differences in details are, of course, inevitable; especially in arsphenamine hypersensitiveness, where considerable variations can be found in the range of specificity in man as well as in guinea pigs. These differences could, undoubtedly, be further reduced, if, in man, higher concentrations of test solutions were to be used than those chosen by Nathan and Grundmann. In principle, in both man and guinea pigs, the skin hypersensitiveness produced by intracutaneous injections of arsphenamine, has been directed *not only to arsphenamines, but, to a lesser degree, also to arsine oxide and aromatic pentavalent arsenicals.* It is most remarkable that analogous observations have been made by Chargin and Leifer (17) in their studies on activation of *fixed arsphenamine eruptions.*

It may be questioned as to whether the arsphenamine hypersensitiveness of guinea pigs and its chemical specificity is worth such painstaking minute examinations. Of course, these experiments have *no direct value in relation to clinical practice.* Antisyphilitic treatment of persons hypersensitive to arsphenamine is not decided by animal experiment but by practical experience. For instance, in spite of the fact that intracutaneous injections of high concentrations of tryparsamide into guinea pigs are followed by local allergic reactions, disturbances produced by the customary antisyphilitic tryparsamide therapy are rare in patients hypersensitive to arsphenamine (see cases of E. Epstein (26); Golz (27); Franks and Fisher (28)).[7]

On the other hand, the eminent practical importance of arsphenamine injuries demands the *most extensive and intensive examinations,* independent of their immediate practical value. Furthermore, there are still many unsolved problems in the field of arsphenamine allergy; and the exact knowledge of the close connections between skin allergy of man and of guinea pigs to arsphenamine, may lead to a *successful approach to the solution of one or another of these problems by animal experiment.* At the same time, these experiments on arsphenamine hypersensitiveness of guinea pigs demonstrate a procedure which

[7] On mapharsen therapy in cases of neoarsphenamine hypersensitiveness see Schoch, Alexander and Long (6c) et al.

should be applied in general to examinations on chemical specificity of allergic skin reactions; the procedure of *cautiously increasing the concentrations* of the test solutions as previously described in this paper.

## SUMMARY AND CONCLUSIONS

Intracutaneous testing of guinea pigs sensitized with old arsphenamine, and of non-sensitized control animals, gave the following results: the skin hypersensitiveness of the sensitized guinea pigs was not directed toward arsenic, nor toward the organic part of arsphenamines, nor exclusively toward arsphenamines, but it was directed in varying degree *toward aromatic arsenicals in general.*

These results are similar to the findings of Frei and R. L. Mayer in some cases of generalized exfoliative neosalvarsan dermatitis, and are almost identical with those of Nathan and Grundmann, obtained in man experimentally sensitized with myosalvarsan. Furthermore, they also correspond to observations made by Chargin and Leifer in their studies on activation of fixed arsphenamine eruptions.

In skin tests on specificity of an allergic state, the concentrations of test substances should be varied within reasonable limits, in order also to embrace low grades of specific affinity.

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